Kentucky Agricultural Experiment Station

University of Kentucky

The Effect of Cod Liver Oil In the Diet of the Mother Hen, On Embryo Mortality and On the Calcium and Phosphorus Content of the Embryo

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were October 1, 1930, to September 30, 1931; October 1, 1931, to September 30, 1932; and October 1, 1933, to September 30, 1934. In 1930-31 and 1933-34, one cockerel was added to each pen at the beginning of the hatching season. Substitutions were made if the males showed lack of vigor or if fertility was low. In 1931-32, cockerels were placed in each pen five days before eggs for incubation were to be collected and remained during the time the eggs were collected. They were then removed and placed in a holding pen with access to bluegrass range. The males were rotated among the pens.

The pullets in the pens were treated as follows:

First pair of pens. Confined to house, sunlight filtered thru common window glass. Pen 1. No cod liver oil. Pen 2. Cod liver oil, 2 percent of the mash.

Second pair of pens. Confined to house with wire-screened, wire-floored sun porch. Pen 3. No cod liver oil. Pen 4. Cod liver oil, 2 percent of the mash.

Third pair of pens. Housed like the first pair of pens but with access to a 50 by 100 feet bluegrass yard. Pen 5. No cod liver oil. Pen 6. Cod liver oil, 2 percent of the mash.

In 1933-34, pens 3 and 4 were not included.

All pens received an all-mash mixture of ground yellow corn 650 pounds, wheat bran 100 pounds, wheat middlings 100 pounds, meat scrap (50% protein) 75 pounds, dried buttermilk 25 pounds, finely ground limestone 20 pounds, steamed bone meal 20 pounds, and salt 10 pounds. Water and coarsely ground limestone (approximately 99 percent calcium carbonate) were available at all times. The calcium content of the mash was 2.15 percent and the phosphorus content 1.24 percent, a calcium to phosphorus ratio of 1.73:1.

In 1930-31 and 1931-32 all eggs laid during the first ten days of each month, from November thru June, were collected for hatching and were set on the eleventh day of each month. In 1933-34, all eggs laid from February 1 thru April 30 were collected and set at weekly intervals. During March and April, 1934, some eggs from each lot were used for the analysis of calcium and phos-

phorus content. All eggs of each setting were incubated in one or the other of two electric cabinet incubators (Petersime or Buckeye) under the standard conditions of temperature and humidity for these incubators. The Petersime incubator was operated at a temperature of 99-¾° F. with a relative humidity of 58% until the eighteenth day. The Buckeye incubator was operated at a temperature of 100° F. with the same humidity as in the other machine. All eggs of each setting were incubated in the same machine. On the eighteenth day of incubation, all eggs were candled, the infertiles and dead germs removed and the remainder transferred to the hatching trays of a Smith electric incubator operated at a temperature of 99° F. and a relative humidity of 70 percent. All eggs that failed to hatch (those that appeared infertile before the candle as well as those containing dead embryos) were broken and observed for development. All dead embryos were compared with previously standardized embryos to determine the age at death.

The eggs used in the study of the calcium and phosphorus content of the embryos were laid during March and April, 1934, and were incubated in a Petersime electric incubator at a temperature of 99-¾° F., with a relative humidity of 58 percent, until the eighteenth day. On the eighteenth day the relative humidity was increased to 70 percent. At intervals of twenty-four hours the embryos from 3 to 7 eggs were removed and compared with previously standardized specimens to make sure that they were normal in size and appearance for the particular day of incubation. They were then freed of all adhering yolk, including that within the body cavity, except the chicks on the twenty-first day, which had completely drawn the yolk into the body cavity. The liquid on the embryos was removed by the use of absorbent paper, the embryos were transferred to silica dishes, weighed and dried to constant weight in an electric oven at 100° C. The dry embryos were burned to a white ash, which was dissolved in hydrochloric acid and the solution diluted to a definite volume with distilled water. Calcium and phosphorus were determined in separate aliquots, each determination being made in duplicate. Calcium was determined by the McCrudden volumetric method (5) and phosphorus by the method of Fiske and Subbarow (6).

PART 1. EFFECT OF COD LIVER OIL ON EMBRYO MORTALITY

Riddle (7) pointed out that, "altho the bird embryo has long been an object of special biological interest as well as of much commercial importance, only a single study has hitherto been made of the age distribution of mortality thruout the period of embryonic life." The study to which he referred was that of Payne (8) who found that in the chicken embryo there are two distinct periods of high mortality, the first on the fourth day and the second on the nineteenth day of incubation. The observations of Riddle (7) on the distribution of mortality for three other avian species, wild doves, ringdoves and common pigeons, are in close agreement with those of Payne, since the peaks of mortality occur at the same relative periods of incubation. Riddle suggested that part of the abnormally high mortality during the early stage is due to failure in respiratory adjustment and that of the later stage to inadequate water supply.

Byerly (9) in a study of his data and those of Payne (8), suggests several possible causes of mortality and reports that the first peak of mortality in the embryos studied in his laboratory occurred on the third day and that there were other peaks on the seventh and tenth days of incubation. Byerly, Titus and Ellis (10) found that the peak of mortality during the second week of incubation was especially pronounced when the hens received a diet containing only vegetable feedstuffs as contrasted with diets containing animal protein concentrates. The same authors (11) reported that, "vacum dried North Atlantic fish meal, steam-dried crab meal, buttermilk, a combination of buttermilk, North Atlantic fish meal and meat meal, and free range were found to enable the production of eggs capable of supporting embryonic life thru the second week of incubation in normal fashion. Diets consisting of certain cereal products supplemented with alfalfa leaf meal, mineral and cod liver oil caused the production of eggs the embryos in which showed a significantly greater mortality during the second week of incubation than the embryos in eggs from birds on a diet supplemented with certain protein concentrates of animal origin."

Smith (12) reported that a diet showing a very low efficiency,

as related to hatchability, during the winter months may be a satisfactory diet for the summer months, because of the action of direct sunshine, and that the amount and quality of sunshine has a very great effect on the first- and second-week peaks in the mortality curve. He found also that lack of vitamin D or an overdosage may produce excessive first- and second-week mortality.

Smith (13) found that as the season progressed, the number of anaemic embryos decreased until they disappeared entirely in July. The blood of these embryos was found to contain only a small amount of hemoglobin. A definite relationship was also found between the amount of sunshine received by the hens and hatchability. Embryo mortality was distinctly lower during the summer than during the winter. The mid-incubation peak of mortality largely disappeared after June, with an accompanying decrease in oedemic, ectopic and chondrodystropic embryos. He concluded that this points strongly toward lack of sunshine as one of the causal agents in the production of these types of abnormalities.

RESULTS AND DISCUSSION

The effect of cod liver oil in the diet, on total embryo mortality, under different conditions, is shown in Table 1. Cod liver oil in the mash of pullets confined to the house and receiving sunshine only thru ordinary window glass decreased embryo mortality 8.7 percent, 34.3 percent and 70.2 percent in the seasons of 1930-31, 1931-32 and 1933-34, respectively. The embryo mortality in the absence of cod liver oil was unusually high; 64.5 percent in 1930-31, 42.8 percent in 1931-32 and 50.0 percent in 1933-34.

Cod liver oil added to the mash of pullets confined to the house and wire-screened, wire-floored sun porch decreased total embryo mortality 19.6 percent in the season of 1930-31 and 4.3 percent in the season of 1931-32.

When pullets were given access to a bluegrass yard, the addition of cod liver oil to the mash decreased total embryo mortality in only one season, 1933-34. In the other two seasons mortality was increased 57.7 percent and 14.2 percent, respectively. Total embryo mortality was lowest in the pens which were allowed access to a bluegrass yard, except in 1930-31.

TABLE 1. Total Embryo Mortality, Percent, and Number of Fertile Eggs Set

Treatment	Season	Mortality, percent		Increase (–|–) or Decrease (–)	Number of fertile eggs	
		No c.l.o.	2% c.l.o.	Percent	No c.l.o.	2% c.l.o.
Confined to house,	1930-31	64.5	58.9	–8.7	293	700
sunshine filtered thru	1931-32	42.8	28.1	–34.3	201	531
window glass	1933–34	50.0	14.9	–70.2	314	690
Confined to house with	1930-31	43.9	35.3	–19.6	716	875
wire-screened, wire–floored sun porch.	1931-32	30.5	29.2	–4.3	321	325
Housed like the first	1930–31	23.9	37.7	–|–57.7	937	1005
pair, but with access	1931–32	21.1	24.1	–|–14.2	697	548
to a bluegrass yard	1933-34	12.7	9.3	–26.8	725	734

As mentioned before, vitamin D is concerned in the assimilation of the bone-forming elements, calcium and phosphorus. This ingredient of the diet, as contained in cod liver oil or as obtained by exposure to sunshine, should have most effect in decreasing mortality of the embryo during the final week of incubation, since calcium and phosphorus assimilation is most rapid during this time. This is borne out by the fact (table 2) that cod liver oil gave no consistent results during the first two weeks of incubation. In some instances there was an increase of mortality and in others a decrease during these periods. Cod liver oil had no consistent effect on mortality the first week either when the pullets were confined to the house, with or without direct sunshine, or when they were allowed bluegrass range. During the second week of incubation there was a decrease in embryo mortality when cod liver oil was given to pullets confined without direct sunshine, and an increase when it was given to confined pullets receiving direct sunshine or to those allowed access to a bluegrass yard with its accompanying direct sunshine.

In all three seasons, under all conditions of the experiment, cod liver oil decreased embryo mortality during the third week of incubation. This decrease was especially pronounced when the pullets were confined to the house. Access to a bluegrass yard with

its accompanying direct sunshine decreased mortality more than direct sunshine alone, when the confined pens are considered as the control.

TABLE 2. Distribution of Embryo Mortality, Percent

Treatment	Season	First Week		Second Week		Third Week	
		No c.l.o.	2% c.l.o.	No c.l.o.	2% c.l.o.	No c.l.o.	2% c.l.o.
Confined to house, sunshine filtered thru window glass	1930-31	9.6	15.0	8.2	8.0	46.8	35.9
	1931–32	15.4	12.6	6.0	4.7	21.4	10.7
	1933-34	9.2	3.8	4.1	0.7	36.6	10.4
Confined to house with wire-screened, wire-floored sun porch	1930-31	9.8	12.0	3.6	6.2	30.5	17.1
	1931-32	11.2	14.2	1.9	3.7	17.5	11.4
Housed like the first pair, but with access to a bluegrass yard	1930-31	6.7	23.4	1.0	2.9	16.2	11.4
	1931–32	10.6	13.0	1.3	2.4	9.2	8.8
	1933-34	6.8	3.7	0.6	1.1	5.4	4.5

To determine the significance of the differences in embryo mortality, the method given by Snedecor (14) for the analysis of variance was used. The results are summarized in Table 3. The mean square within the pen within the week is nearly the same for each of the three years, indicating a rather constant source of uncontrolled variation (experimental error).

In all three years, the mean square between the pens is highly significant. The variance between pens within the week is highly significant in the 1930-31 and 1933-34 seasons, but is just significant in the 1931-32 season (eliminating the variation from week to week). This shows that the treatment to which the hens were subjected, significantly influenced embryo mortality. The variance in embryo mortality between the weeks is highly significant, likewise the mean square between weeks within the pen is highly significant (eliminating influence of treatment). The magnitude of the sum of squares between the weeks is much greater than that between the weeks within the pen. This indicates that the treatments to which the hens were subjected did not influence embryo mortality equally in all three weeks. That such was the case is shown in Figure 1.

TABLE 3. Analysis of Variance of Chick-Embryo Mortality

Source of Variation	D /F	Sum of Squares	Mean Square	F
1930-31				
Total	137	25,300.	185.	
Between Pen-Week Groups	17	19,107.	1,124.**	22.2
Between Pens	5	2,971.	594.**	11.7
Between Weeks	2	10,285.	5,143.**	101.6
Between Pens Within Week	15	8,822.	588.**	11.6
Between Weeks Within Pen	12	16,137.	1,345.**	26.6
Within Pen Within Week	120	6,193.	51.	
1931–32				
Total	143	9,732.	68.	
Between Pen-Week Groups	17	4,242.	250.**	5.7
Between Pens	5	823.	165.**	3.8
Between Weeks	2	2,927.	1,463.**	33.6
Between Pens Within Week	15	1,315.	88.*	2.0
Between Weeks Within Pen	12	3,419.	285.**	6.5
Within Pen Within Week	126	5,490.	44.	
1933–34				
Total	143	18,293.	128.	
Between Pen-Week Groups	35	12,231.	349.**	7.6
Between pens	5	4,666.	933.**	20.3
Between Weeks	2	3,939.	1,970.**	42.9
Between Pens Within Weeks	9	8,292.	921.**	20.1
Between Weeks Within Pen	8	7,565.	946.**	20.6
Within Pen Within Week	132	6,061.	46.	

* Significant
** Highly Significant

PART 2. EFFECT OF COD LIVER OIL ON THE CALCIUM AND PHOSPHORUS CONTENT OF THE EMBRYO

A study of the literature on the growth of the chick embryo reveals that the deposition of calcium in the embryo is greatest during the last few days of incubation. An extensive review of this phase of embryonic life is given by Needham (15). The observations of Hart, Scott, Kline and Halpin (16) are of special importance. They found a gradual transfer of calcium from the shell to the growing embryo and a marked shift in the ratio of calcium to phosphorus as incubation proceeds.

With an initial Ca:P ratio of 1:2.77 and an almost constant percentage of phosphorus in the dried contents of the egg, the ratio on the twentieth day of incubation was 1:0.78, with an increase of calcium from 0.27 percent to 1.14 percent.

Buckner, Martin and Peter (17) found that the quantity and percentage of phosphorus were approximately the same in strong and in weak chicks but that the quantity and percentage of calcium were less in weak than in strong chicks.

Hart and coworkers (18) reported that the calcium content of chicks from eggs produced by irradiated hens was appreciably greater than that of chicks from eggs of non-irradiated hens. Unfortunately these workers failed to make a simultaneous study of embryo mortality under these conditions.

RESULTS AND DISCUSSION

Since it seems that vitamin D is directly concerned with the assimilation of bone-forming elements, notably calcium and phosphorus, during the final week of incubation, a study of these constituents should lead to a better understanding of the role played by this vitamin in avian embryonic life. The data on the calcium content of the chick embryo as affected by the various treatments to which their dams were subjected are presented in Tables 4, 5 and 6, and figures 2 and 3. The total calcium content of the fifteenth to the twenty-first day embryos from the pullets which were confined without cod liver oil is consistently lower than that of the embryos from those which were confined but received two percent cod liver oil in the mash. These differences are apparent when the calcium is calculated as percentage of the dry embryo or as percentage of egg weight. (The number of embryos analyzed,

the average egg weight and average weight of oven dried embryo are given in Table 8).

TABLE 4. Calcium and Phosphorus In the Oven Dried Embryo

Treatment	Day of Incubation	Calcium, percent,		Phosphorus, percent	
		No c.l.o.*	2% c.l.o.	No c.l.o.	2% c.l.o.
Confined to house, sunshine filtered thru window glass	15	0.79	0.94	1.31	1.54
	16	0.82	0.97	1.14	1.20
	17	0.93	1.20	1.13	1.25
	18	1.11	1.41	1.11	1.29
	19	1.18	1.44	1.16	1.31
	20	1.37	1.55	1.28	1.38
	21	1.07	1.34	0.96	1.01
Housed like the other pair, but with access to a bluegrass yard	15	0.85	0.78	1.39	1.20
	16	0.92	0.89	1.17	1.14
	17	1.07	1.10	1.21	1.24
	18	1.28	1.35	1.23	1.30
	19	1.52	1.50	1.33	1.29
	20	1.52	1.59	1.31	1.32
	21	1.30	1.39	1.03	1.03

* c. l. o.—cod liver oil.

TABLE 5. Calcium and Phosphorus in the Embryo, as Percentage of the Egg Weight

Treatment	Day of Incubation	Calcium, percent,		Phosphorus, percent	
		No c.l.o.*	2% c.l.o.	No c.l.o.	2% c.l.o.
Confined to house, sunshine filtered thru window glass	15	.002	.003	.004	.004
	16	.003	.005	.005	.006
	17	.006	.007	.007	.008
	18	.008	.011	.008	.010
	19	.010	.013	.010	.012
	20	.013	.015	.012	.013
	21	.019	.024	.017	.018
Housed like the other pair, but with access to a bluegrass yard	15	.002	.003	.004	.005
	16	.004	.005	.006	.006
	17	.007	.007	.008	.008
	18	.010	.009	.010	.008
	19	.013	.014	.011	.012
	20	.016	.016	.014	.013
	21	.023	.025	.018	.018

* c. l. o.—cod liver oil.

TABLE 6. Average Weight of Calcium and Phosphorus Per Embryo

Treatment	Day of Incubation	Calcium, grams,		Phosphorus, grams	
		No c.l.o.*	2% c.l.o.	No c.l.o.	2% c.l.o.
Confined to house,	15	.015	.014	.025	.023
sunshine filtered	16	.020	.025	.028	.031
thru window glass	17	.033	.041	.040	.043
	18	.043	.058	.043	.053
	19	.054	.069	.053	.063
	20	.075	.082	.070	.073
	21	.107	.133	.096	.100
Housed like the	15	.014	.015	.023	.023
other pair, but	16	.025	.025	.032	.032
with access to a	17	.040	.039	.045	.044
bluegrass yard	18	.057	.054	.055	.052
	19	.072	.074	.063	.064
	20	.094	.090	.081	.075
	21	.134	.130	.106	.097

* c. l. o.—cod liver oil.

It should be noted, also, that the calcium content of the embryos from the group which had bluegrass range was higher on the nineteenth and twentieth days of incubation than that of the embryos from the confined group. Differences are not so apparent on the twenty-first day of incubation, but the embryos from the two pens that had bluegrass range and the confined pen which received cod liver oil contained a decidely larger quantity of calcium than the embryos from the confined pen without vitamin D supplement. When considered as percentage of the dry embryo or of the egg weight, the same relationship exists between the confined pen with no supplement and the other three pens. There is not a very apparent difference between the latter three pens on the nineteenth and twentieth days.

The phosphorus content of the embryos is shown in Tables 4, 5 and 6, and Figures 2 and 3. These results present essentially the same picture as do those for the calcium content of the embryos. The embryos from eggs of the pullets which were confined without cod liver oil contain appreciably less phosphorus than those from the confined pullets receiving cod liver oil, or from pullets allowed bluegrass range either with or without the addition of

cod liver oil. Again, the differences are most noticeable on the eighteenth and nineteenth days of incubation. As pointed out before, these differences may be directly associated with the peak of embryo mortality which occurs on the nineteenth day of incubation. It would seem that any interference with the normal metabolism of calcium and phosphorus might lead, thru weakness in the bone-forming ability of the embryo, to an increase in the

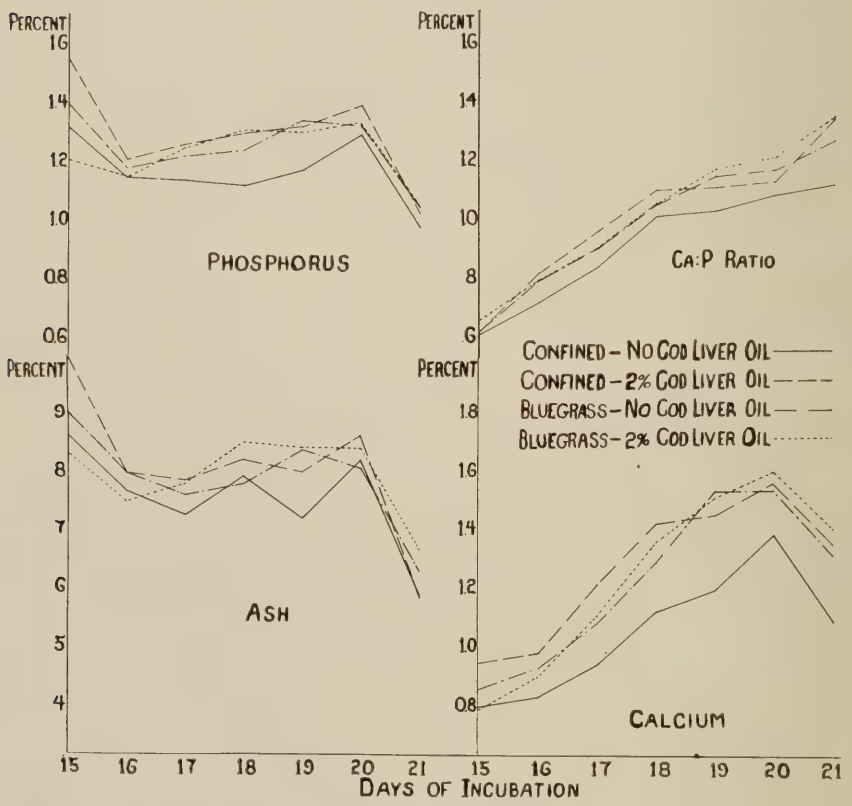

Fig. 2. Total ash, calcium, and phosphorus as percent of the dry embryo, and ratio of calcium to phosphorus.

final peak of mortality which occurs on the nineteenth day of incubation.

Differences in the phosphorus content of the embryos are also noticeable when the phosphorus is expressed as percentage of the dry embryo or of egg weight. Again, the greatest differences be-

tween the confined pen without cod liver oil and the other three groups occur on the eighteenth, nineteenth and twentieth days, which is, as mentioned above, a decidedly critical period in embryonic life. Calcium and phosphorus analyses on a smaller number

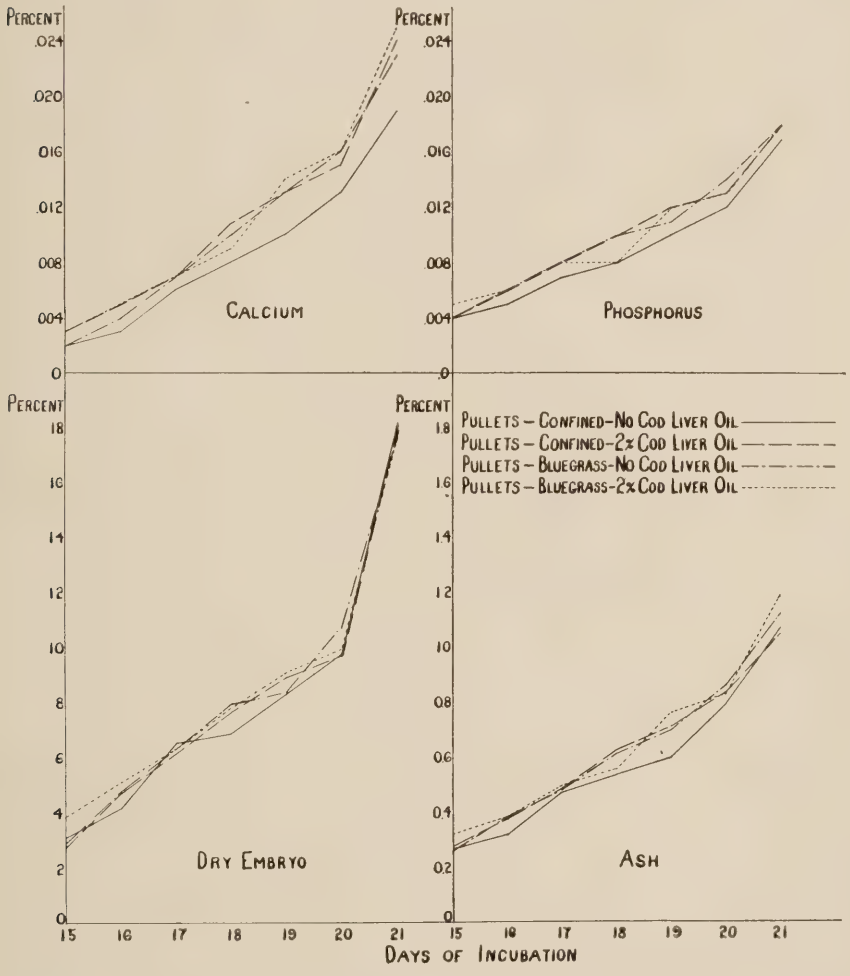

Fig. 3. Dry embryo, total ash, calcium, and phosphorus as percentage of egg weight.

of embryos from the same pens during the 1931-32 season are in accord with those recorded here.

Calcium and phosphorus content of the embryos at hatching time was consistently smaller when there was no vitamin D supplement in the diet of the pullets that produced the eggs. In ad-

dition to possible weakness in skeletal formation, chicks from eggs produced by hens under these conditions may be so weakened in other respects that mortality may be especially high during the first few weeks after hatching. Unpublished data at this Station show that when these chicks are grown, either in confinement or with range and with the addition of cod liver oil to their mash, they manifest an abnormally high mortality.

Buckner, Martin and Peter (17), using eggs produced by hens receiving a normal ration, found that the quantity and the percentage of calcium were less in weak than in strong chicks, while the phosphorus content was approximately the same in both. In the present experiment, however, the lack of a sufficient quantity of vitamin D tended to reduce the quantity of both bone-forming elements in the embryo.

It is evident from the data presented in Table 7 and Figures 2 and 3, that the total ash content of the sixteenth to twentieth day embryos from the hens which were confined without cod liver oil is smaller than that of the embryos from hens which were confined but which received two percent of cod liver oil in the mash.

TABLE 7. Ash in the Embryo

Treatment	Day of Incubation	As Percentage of Dry Embryo		As Percentage of Egg Weight	
		No c.l.o.*	2% c.l.o.	No c.l.o.	2% c.l.o.
Confined to house, sunshine filtered thru window glass	15	8.59	9.93	0.266	0.274
	16	7.63	7.95	0.316	0.378
	17	7.23	7.81	0.473	0.484
	18	7.89	8.18	0.539	0.625
	19	7.16	7.94	0.593	0.708
	20	8.15	8.58	0.792	0.836
	21	5.87	5.80	1.067	1.049
Housed like the other pair, but with access to a bluegrass yard	15	8.97	8.28	0.260	0.322
	16	7.95	7.44	0.382	0.381
	17	7.56	7.75	0.481	0.495
	18	7.76	8.47	0.616	0.552
	19	8.32	8.38	0.698	0.761
	20	8.03	8.34	0.860	0.825
	21	6.27	6.62	1.123	1.183

* c. l. o.—cod liver oil.

These differences in ash content are likewise apparent when expressed as percentage of the dry embryo or of the weight of the egg. The smaller ash content of the embryos from the eggs produced by the confined hens receiving no cod liver oil is particularly noticeable on the eighteenth and nineteenth days. The values of the eighteenth day are 0.306 grams of ash for the embryos from the confined pen with no cod liver oil and 0.336 for the confined pen with two percent cod liver oil. For the nineteenth day the ash content is 0.328 for the first group and 0.381 for the second group. It is interesting, and perhaps significant, that there is a peak of mortality on the nineteenth day of incubation.

There is no great difference between the ash content of the embryos from the pens which were allowed bluegrass range, either with or without cod liver oil, or between the ash content of the embryos from either of these two groups and that of the embryos from the pullets confined with two percent of cod liver oil in the mash.

The apparently rapid increase in the ash content of the embryos from the twentieth to the twenty-first day, when considered as percentage of egg weight, is due to the fact that the yolk was not removed from the body cavity on the twenty-first day, since it had already been drawn into the body and the opening healed.

TABLE 8. Average Egg Weight, Number of Embryos Analyzed and Average Oven-Dry Weight of Embryos—1933-34

Day of Incubation	Average weight of eggs, grams.				No. of embryos analyzed.				Embryo dry weight, grams.			
	Confined		Bluegrass range		Confined		Bluegrass range		Confined		Bluegrass range	
	No. c.l.o.	2% c.l.o.	No. c.l.o.	2% c.l.o.	No. c.l.o.	2% c.l.o.	No. c.l.o.	2% c.l.o.	No. c.l.o.	2% c.l.o.	No. c.l.o.	2% c.l.o.
15	61.6	54.0	56.9	49.4	4	5	5	5	1.91	1.49	1.65	1.92
16	59.1	54.3	56.8	54.8	6	6	7	7	2.45	2.58	2.73	2.81
17	54.1	55.4	58.6	55.6	5	7	7	7	3.54	3.43	3.73	3.55
18	56.8	53.8	56.2	61.2	6	7	6	6	3.88	4.11	4.46	3.99
19	55.3	53.8	56.6	54.5	6	7	6	6	4.58	4.80	4.75	4.95
20	56.3	54.4	57.8	57.3	6	5	5	6	5.47	5.30	6.19	5.67
21	55.1	54.8	57.7	52.5	4	4	4	3	10.01	9.92	10.33	9.38

* c. l. o.—Cod Liver Oil.

SUMMARY

A study was made of the influence of cod liver oil in the ration of White Leghorn pullets on embryo mortality under three sets of conditions: (1) confined to the house, lighted thru ordinary window glass, (2) confined to the house, but allowed direct sunshine, and (3) allowed bluegrass range. The influence of cod liver oil on the calcium and phosphorus content of the embryo under the first and third set of conditions, was also studied.

Pullets confined without vitamin D supplement produced eggs showing a very high embryo mortality during the third week of incubation. Exposure of the pullets to sunshine or allowing them bluegrass range very markedly reduced the embryo mortality during this period. Bluegrass range was more effective than sunshine alone. The addition of cod liver oil to the mash of pullets under these three sets of conditions decreased embryo mortality during the third week. The decrease was most marked in the confined pen, less in the sunshine pen and only a very slight decrease in the bluegrass range pen.

The calcium and phosphorus content of the embryos from the pen which was confined without antirachitic treatment was consistently less after the sixteenth day than that of the embryos from the other pens. This difference is apparent when the calcium and phosphorus is calculated as percentage of the egg or as percentage of the dry embryo. It is perhaps significant that the greatest difference in calcium and phosphorus content between the embryos from the pen which was confined without antirachitic treatment and that of the embryos from the other three pens, occurred on the eighteenth and nineteenth day of the incubation period which coincides with the peak of embryo mortality during the last week of incubation.

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